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The use of honeydew in foraging for aphids by larvae of the ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)

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Abstract. 1. To clarify the use of honeydew in foraging for aphids by larvae of the ladybird beetle, *Coccinella septempunctata* L., searching behaviour of ladybird larvae for *Aphis craccivora* Koch and *Acyrthosiphon pisum* Harris and the abundance of honeydew under aphid colonies were examined in laboratory experiments.

2. More larvae responded by climbing the plants with aphids than responded to plants without aphids. When the plants were replaced with sticks, in order to exclude visual and olfactory cues from plants and aphids, more larvae of *C. septempunctata* climbed sticks above the area that contained honeydew than climbed sticks above the area that did not contain honeydew. Then, ladybird larvae use honeydew as a contact kairomone when foraging for aphids.

3. *Aphis craccivora* deposited a larger number of honeydew droplets beneath the plants than did similar numbers of *A. pisum.* Thus, *C. septempunctata* larvae licked more frequently the honeydew of *A. craccivora* than that of *A. pisum* and spent longer searching on the area containing honeydew of *A. craccivora* than that of *A. pisum*. Consequently, a larger number of larvae climbed a stick above honeydew of *A. craccivora* than that of *A. pisum.*

4. It may be also considered that *C. septempunctata* larvae can distinguish honeydew of the two aphid species and respond more strongly to *A. craccivora* than *A. pisum.*

Key words. *Acyrthosiphon pisum*, ant attendance, *Aphis craccivora*, *Coccinella septempunctata*, foraging behaviour, honeydew.

Introduction

It is well known that adult coccinellids are strongly attracted to sites having an abundance of aphids (Hodek & Honek, 1996), and that they access aphids using various chemical cues. For example, adults of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) are attracted to the odour of aphid-infested leaves (Obata, 1986, 1997), and adults of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) are attracted to (E)-β-farnesene, which is a component of the alarm pheromone of many aphid species, particularly in the subfamily Aphidinae (Al Abassi *et al*., 2000). On the other hand, it has been considered that ladybird larvae search for prey nearly at random and that they are unable to detect prey before physical contact (Banks, 1954; Kehat, 1968). However, Stubbs (1980) demonstrated larval responses to the odours of crushed prey and inferred a role of olfaction in prey recognition. Jamal and Brown (2001) also suggested that ladybird larvae use olfactory cues in foraging, much as adults do.

Aphids suck phloem sap from plants and excrete honeydew. Carter and Dixon (1984) reported that *C. septempunctata* larvae searched for a longer time on a plant with aphid honeydew than on a plant without honeydew. Thus, it was hypothesised that honeydew could be an important cue used by *C. septempunctata* larvae when searching for prey.

Coccinella septempunctata larvae frequently feed on aphids of *Aphis craccivora* Koch and *Acyrthosiphon pisum* Harris (Homoptera: Aphididae) in western Japan where these two aphid species frequently colonise on leguminous plants such as

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the broad bean *Vicia faba* L. and the vetch *Vicia angustifolia* L. (Suzuki *et al*., 2004).

Aphis craccivora is an ant-tended aphid that can gain protection from ants attracted to its honeydew (Katayama & Suzuki, 2002, 2003). The mutualism between *A. craccivora* and ants is facultative and the aphids are not always tended by ants, but *A. craccivora* rarely shows escape responses to its natural enemies such as ladybird larvae, even when ants are absent. On the other hand, *A. pisum*, a species not tended by ants, tend to drop from a host plant to escape predation (Kislow & Edwards, 1972; Nault *et al*., 1973). Therefore, in the absence of ants, *C. septempunctata* larvae can feed more efficiently on *A. craccivora* than on *A. pisum* (Ide, 2006).

The honeydew excreted by aphids contaminates peripheral plant parts such as the surface of leaves and stems surrounding aphid colonies. Accumulations of honeydew on the surfaces of plants frequently pose a risk to the aphids themselves because of its physical properties (Buckley, 1987). Therefore, aphids of many species, including *A. pisum*, flick their droplets of honeydew as far away as possible with a raised abdomen, or kick the droplets off with a hind leg (Way, 1963). On the other hand, ant-tended *A. craccivora* aphids drops the honeydew at their feet without flicking or kicking because it is usually removed by their tending ants. Thus, it might be expected that accumulation of honeydew under aphid colonies to differ between ant-tended and non-ant-tended species. If ladybird larvae search for aphids using honeydew as a cue, they may detect ant-tended and non-ant-tended aphid colonies with different probabilities.

To test these expectations, the present study examined the behaviour of *C. septempunctata* larvae searching for *A. craccivora* and *A. pisum* in laboratory experiments. In particular, the following questions were addressed: (1) Do *C. septempunctata* larvae prefer plants infested with *A. craccivora* or *A. pisum* to those without aphids? (2) Do *C. septempunctata* larvae use the honeydew residues of *A. craccivora* or *A. pisum* to locate prey? (3) Does the species of an aphid colony affect its probability of discovery by *C. septempunctata* larvae?

Materials and methods

Choice test with plants

Colonies of *A. craccivora* and *A. pisum* were established from individuals collected from *V. angustifolia* plants on the campus of Saga University, Saga City, western Japan (33°14′N, 130°18′E). Aphids of each species were separately reared on seedlings of *V. faba* in the plastic cages (length: 32 cm, width: 25 cm, height: 25 cm) with a nylon mesh at the top at 20 °C under L:D 12:12 h or L 24 h photoperiod conditions in a growth chamber.

Adult females of *C. septempunctata* were collected on the campus of Saga University and were held individually in the Petri dishes (9 cm in diameter, 1.5 cm in depth) with equal amounts of *A. craccivora* and *A. pisum* supplied daily as food. All insects were held in a growth chamber at 20 °C under L:D 12:12 h photoperiod in the laboratory. Eggs of *C. septempunctata* were obtained from oviposition by these adults, and were incubated in a growth chamber. Hatched larvae were singly reared in the Petri dishes (as above) with approximately equal amounts of *A. craccivora* and *A. pisum* supplied daily as food. The fourth-instar larvae used in experiments were fed only water for 6 h prior to testing in order to increase their hunger level and stimulate searching behaviour.

Seedlings of *V. faba* were grown in polyethylene pots (9 cm in diameter, 6.5 cm in depth) at 20 °C under L:D 14:10 h photoperiod condition. Plastic pots (10 cm in diameter and 4.5 cm in depth) were filled with water and covered with a Petri dish lid with a 1.5 cm hole in the centre. The stem of each *V. faba* seedling with three leaf nodes (each leaf node with two leaves) was pushed through the hole. The base of the seedling was covered with wet cotton wool to prevent it from drying and to stabilise the stem.

One hundred aphids of *A. craccivora* or *A. pisum* were released on each *V. faba* plant. After 24 h, one plant with aphids and another plant without aphids were placed together on an experimental apparatus (length: 19 cm, width: 25 cm, height: 9 cm) covered with plaster to a depth of 5 cm with two holes in which to set the plastic pots (Fig. 1a). The inner sides of the apparatus walls were coated with talc powder to prevent larvae from escaping.

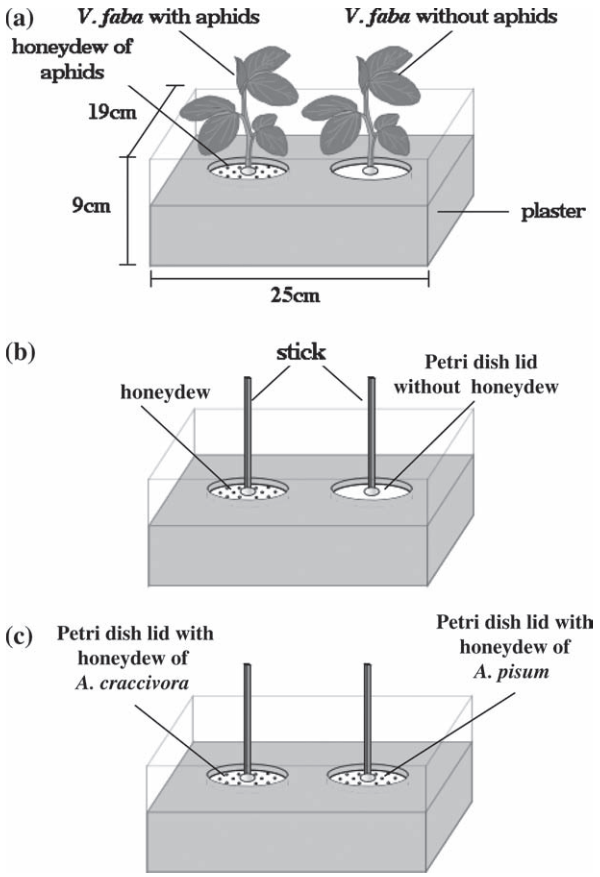

Fig. 1. Illustrations of the experimental apparatus in the experiments concerning choice test with plants (a), responses to honeydew alone (b), and honeydew choice test (c).

The experiment was carried out at 25 °C under a fluorescent light (27W) on a laboratory bench. Fourth-instar larvae of *C. septempunctata* were released singly in the centre of the experimental apparatus. The experiment was replicated 22 times with *A. craccivora*-infested plants and 22 times with *A. pisum*-infested plants. The apparatus was washed with water after each replicate and all plants were used only once. The number of *C. septempunctata* larvae arriving at Petri dish lids under the plants with and without aphids was counted, as well as the number of *C. septempunctata* larvae that climbed the plants with and without aphids. Each replicate was terminated when the larva first climbed a plant, or after 30 min without a response.

Responses to honeydew alone

One hundred aphids of either *A. craccivora* or *A. pisum* were released on a *V. faba* plant and left to deposit honeydew on a Petri dish lid for 24 h at 20 °C under L:D 12 h:12 h photoperiod condition. After 24 h, the Petri dish lid with honeydew and another clean lid without honeydew were both set on the experimental apparatus and a wooden stick (diameter: 5 mm, length: 15 cm) was pushed through the holes at the centre of each lid. The base of the wooden stick was covered with cotton wool to stabilise the stick (Fig. 1b).

The experiment was carried out under the same environmental conditions as the choice test. Fourth-instar larvae of *C. septempunctata* were released one at a time in the centre of the apparatus until 22 replicates were performed with each aphid species. The apparatus was washed with water between replicates and each stick was used only once. The number of *C. septempunctata* larvae arriving at the Petri dish lids with and without honeydew was counted, as well as the number of *C. septempunctata* larvae that climbed the sticks above the lids. Each replicate was terminated when a larva climbed a stick, or after 30 min without a response.

Honeydew choice test

Petri dish lids coated with the honeydew of each aphid species were prepared as described in the previous experiment. Lids containing the honeydew of *A. craccivora* and *A. pisum* were set in pairs on the experimental apparatus, and a wooden stick was pushed through the centre of each Petri dish lid as described above (Fig. 1c). Fourth-instar larvae of *C. septempunctata* were released singly in the centre of the apparatus. Sticks were used only once and the apparatus was washed with water between trials until a total of 66 replicates were performed. The number of *C. septempunctata* larvae arriving at Petri dish lids containing honeydew of each aphid species was counted as was the number that climbed sticks above lids. Also tallied was the number of times each larvae licked the honeydew of each aphid species and its total residence time on the lid. The experiment was terminated when a larva climbed a stick, or after 30 min without a response.

Honeydew deposition

One hundred aphids of either *A. craccivora* or *A. pisum* were released on each of a series of *V. faba* plants and allowed to deposit honeydew on Petri dish lid under the same conditions as above. The number of honeydew droplets on each lid was determined by dividing the 36 cm^2 area of the lid into 36 sections of 1 cm^2 and counting the number of droplets within each. The experiments were replicated 18 times for each aphid species.

Statistical analysis

The results of the choice test with plants, responses to honeydew alone, and the honeydew choice test were analysed using a binomial test. Data on the foraging behaviour of larvae and the deposition patterns of honeydew on Petri dish lids were compared using a Mann–Whitney *U*-test.

Results

Choice test with plants

The number of *C. septempunctata* larvae arriving at Petri dish lids under the *V. faba* plants did not differ between plants with and without aphids (binomial test, *A. craccivora*: $n = 22$, $P = 0.2617$; *A. pisum*: $n = 22$, $P = 0.4159$; Fig. 2a,b). However,

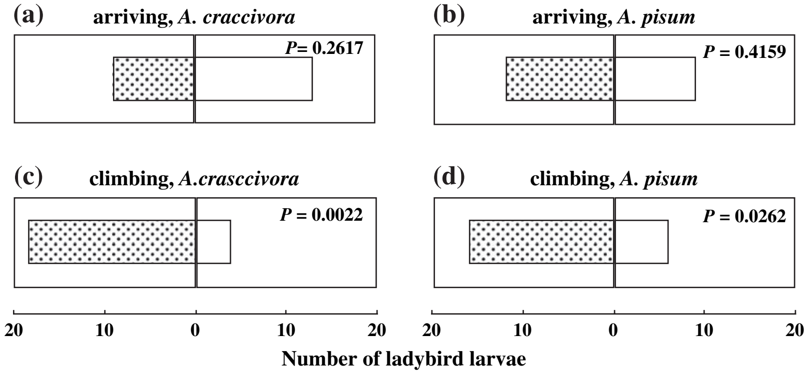

Fig. 2. The number of *Coccinella septempunctata* larvae arriving at Petri dish lids under plants with and without aphids of *Aphis craccivora* (a) and *Acyrthosiphon pisum* (b), and climbing plants with and without aphids of *A. craccivora* (c) and *A. pisum* (d) in choice test with plants. Solid and open columns indicate plants with and without aphids respectively.

more *C. septempunctata* larvae climbed plants with aphids than climbed plants without aphids (binomial test, *A. craccivora*: $n = 22$, $P = 0.0022$; *A. pisum*: $n = 22$, $P = 0.0262$; Fig. 2c,d).

Responses to honeydew alone

The numbers of ladybird larvae arriving at Petri dish lids did not differ between those that did or did not contain honeydew (binomial test, honeydew of *A. craccivora*: $n = 22$, $P = 0.2617$; honeydew of *A. pisum*: $n = 22$, $P = 0.5841$; Fig. 3a,b). However, more *C. septempunctata* larvae climbed sticks above lids that contained honeydew than climbed sticks above those that did not (binomial test, *A. craccivora*: $n = 22$, $P = 0.0004$; *A. pisum*: $n = 22$, $P = 0.0022$; Fig. 3c,d).

Honeydew choice test

Similar numbers of *C. septempunctata* larvae arrived at Petri dish lids containing honeydew of either *A. craccivora* or *A. pisum* (binomial test, $n = 66$, $P = 0.5483$; Fig. 4a). However, more *C. septempunctata* larvae climbed sticks above lids containing honeydew of *A. craccivora* than climbed sticks above lids containing honeydew of *A. pisum* (binomial test, $n = 66$, $P = 0.0175$; Fig. 4b).

Honeydew deposition and consumption by larvae

Honeydew consumption behaviour of *C. septempunctata* larvae is shown in Table 1. A larger proportion of *C. septempunctata* larvae licked honeydew of *A. craccivora* than licked honeydew of *A. pisum*, there was a greater number of licking events, and larval residence times were significantly longer on lids containing *A. craccivora* honeydew. Honeydew deposition by *A. craccivora* resulted in more than twice the density of droplets per unit area on Petri dish lids compared with that of *A. pisum* [*A. craccivora*: $n = 18\,501.72 \pm 17.55$ (mean ± SE); *A. pisum*: $n = 18\,205.56 \pm 11.34$ Mann–Whitney *U*-test, $P < 0.0001$].

Discussion

More larvae of *C. septempunctata* climbed the plants bearing aphids than climbed those without aphids (Fig. 2c,d). This result suggests that *C. septempunctata* larvae gather the information concerning the presence of aphids on plants before climbing them. Although many authors have reported that ladybird adults use visual or olfactory cues derived from plants or aphids in their foraging (Nakamuta, 1984; Obata, 1986; van den Meiracker *et al.*, 1990; Nakamuta, 1991; Obata, 1997; Harmon *et al.*, 1998; Al Abassi *et al.*, 2000; Ninkovic *et al.*, 2001), cues in the foraging behaviour of ladybird larvae have scarcely been investigated (Carter & Dixon, 1984; Jamal & Brown, 2001).

Although the experimental design in the present study did not eliminate the possibility that *C. septempunctata* larvae use a visual cues in their foraging, olfactory cues were not implicated because larvae arrived with similar frequencies at Petri dish lids under plants with and without aphids (Fig. 2a,b). The experiment involving honeydew alone effectively excluded visual and olfactory cues associated with either plants or aphids and showed that more larvae of *C. septempunctata* climbed sticks above the area that contained honeydew than climbed sticks above the area that did not contain honeydew (Fig. 3c,d). Although it has been reported that many coccinellid species feed on honeydew (Hagen, 1962), this study showed that ladybird larvae use honeydew as a contact kairomone when foraging for aphids.

Carter and Dixon (1984) observed that *C. septempunctata* larvae spent a longer time on plants soiled with honeydew than those that were not. Murakami and Tsubaki (1984) reported that *C. septempunctata* larvae executed a linear search for their prey, but changed to an area-concentrated search following prey encounter. In this study, *C. septempunctata* larvae also switched from a linear search to an area-concentrated one after licking honeydew and subsequently scaled a plant or a stick.

As mentioned above, the non-ant-tended species *A. pisum* flicks droplets of honeydew as far as possible with the tip of abdomen raised, or kicks the droplets off with a hind leg. In contrast, the ant-tended *A. craccivora* does not invest in such efforts because tending ants normally remove their honeydew. In the present study it was demonstrated that this difference in

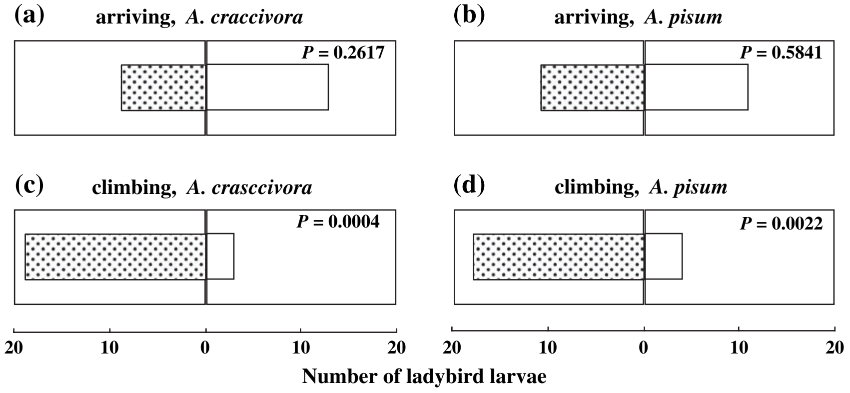

Fig. 3. The number of *Coccinella septempunctata* larvae arriving at Petri dish lids that did and did not contain honeydew of *Aphis craccivora* (a) and *Acyrthosiphon pisum* (b), and climbing sticks above Petri dish lids that did and did not contain honeydew of *A. craccivora* (c) and *A. pisum* (d) in the experiment concerning responses to honeydew alone. Solid and open columns indicate Petri dish lids that did and did not contain honeydew respectively.

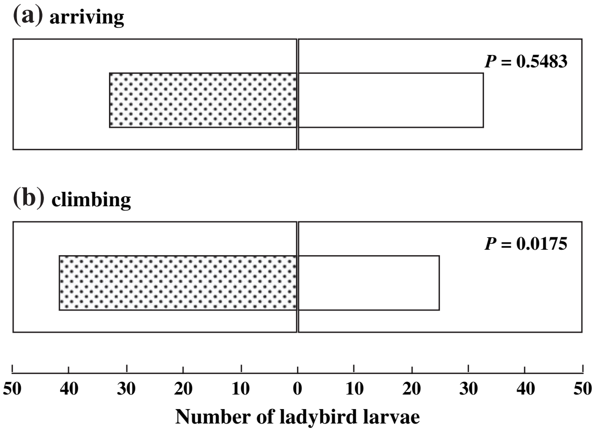

Fig. 4. Choice of honeydew of two aphid species by *Coccinella septempunctata* larvae. (a) The number of *C. septempunctata* larvae arriving at Petri dish lids containing honeydew of *Aphis craccivora* (solid column) and *Acyrthosiphon pisum* (open column). (b) The number of *C. septempunctata* larvae climbing sticks above a Petri dish lids containing honeydew of *A. craccivora* (solid column) and *A. pisum* (open column).

behaviour between an ant-tended and a non-ant-tended aphids species influences the abundance of honeydew under aphid colonies, and consequently the probability of discovery by *C. septempunctata* larvae. The density of *A. craccivora* honeydew on a Petri dish lid was higher than that of *A. pisum*, resulting in more licking by *C. septempunctata* larvae and a longer period of area-restricted search (Table 1). Consequently, more *A. craccivora* colonies were 'discovered' by *C. septempunctata* larvae than were those of *A. pisum* (Fig. 4). This finding also suggests that ant-tended aphids might be more vulnerable to discovery by *C. septempunctata* larvae than non-ant-tended aphids when they are deprived of the services of ants.

Carter and Dixon (1984) reported that when aphids were present, the amount of honeydew on a leaf did not affect the residence time of a *C. septempunctata* larva on a leaf. In the context of the results of the present study, this observation might be due to the fact that larvae cease responding to honeydew once aphids are encountered.

Völkl *et al*. (1999) and Fischer and Shingleton (2001) reported that the sugar composition of honeydew differed between ant-tended and non-ant-tended aphids. Thus, it is also possible that *C. septempunctata* larvae can distinguish between the honeydews of these two aphid species and responded more strongly to that of *A. craccivora* compared with that of *A. pisum* on the basis of qualitative, as well as quantitative, differences.

Table 1. The honeydew consumption behaviour of ladybird larvae in the experiment concerning the choice of honeydew of *Aphis craccivora* and *Acyrthosiphon pisum* .

	A. craccivora ($n = 48$)	*A. pisum* ($n = 40$)	*P**
Proportion of ladybird larvae that licked honeydew on a Petri dish lid	0.76 ± 0.06	0.54 ± 0.07	0.0241
Number of times ladybird larvae licked honeydew on a Petri dish lid	4.86 ± 0.58	2.29 ± 0.47	0.0011
Residence time on a Petri dish (s)	447.35 ± 50.99	322.62 ± 47.39	0.0396

*Mann–Whitney *U*-test.

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